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GENETICS OF THE ENCEPHALITIS VECTOR, 'CULEX TARSALIS' FOR POSSI--ETC(U)  
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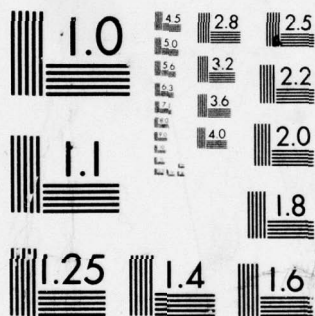
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The projects represent part of an overall program designed to change <u>Culex</u> <u>tarsalis</u> genetically to inhibit its propagation in nature, and to render it less effective as a vector of disease. The study has two principle objectives: 1.) the use of reciprocal translocations that would serve as self-destroying systems when introduced into a native population and/or function as vehicles of transport to carry desirable genotypes into the population; 2.) the de- velopment of genotypes that when introduced into a population would contribute		

(20. continued)

to its reduction and/or its inability to act as a successful vector of WEE and SLE virus. Mutant lines have been isolated and are currently being combined into multiple-marker stocks. Several induced translocations have been identified cytologically and are established as stocks. Once multiple marker lines are functional, methods will be available to identify and isolate translocations by genetic means. Desirable genotypes now being investigated that could be carried by introduced homozygous interchanges are: mutants that contribute to lethality, and genomes that render the mosquito refractory to infection.

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REPORT NO. USA-A74-75

GENETICS OF THE ENCEPHALITIS VECTOR, CULEX TARSALIS FOR  
POSSIBLE APPLICATION IN INTEGRATED CONTROL

Annual 1974-75 (First Year)

Sister Monica Asman, Ph.D.

March 30, 1975

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## RESUME OF PROGRESS TO DATE

### I. Abstract of significant developments on Culex tarsalis genetics.

- a. Ten new mutant strains have been isolated that may have application for mapping of chromosomes and as possible tracer stocks for future field programs directed at population control.
- b. Seven lines that carry different induced translocations have been established.
- c. A line that carries a genetic factor responsible for polyploidy and sperm lethality has been isolated.
- d. The selection for genetic lines refractory to Western Encephalitis virus and of lines unable to transmit virus have progressed well.
- e. Studies have been initiated on the genetics of diapause and autogeny.
- f. Two experiments have been completed--one relating to mating behavior and one relating to using eggs as a holding stage for this species. These will be submitted for publication in February, 1975, as Scientific Notes to Ann. Ent. Soc. Amer. and Mosquito News respectively.
- g. Two other papers---Four new eye mutants in Culex tarsalis and Radiation-induced reciprocal translocations in Culex tarsalis will be completed for publication within the next (3rd) quarter.  
The principal investigator was invited to discuss her current research at two symposiums, one at the California Mosquito Control Association meeting and the other at the Entomological Society of America meeting. Her associate, Dr. Paul McDonald, also gave invitational papers at these meetings.

### II. Experimental

#### A. Culex tarsalis strains and "marker" lines.

Considerable time has been given these first 2 quarters to finding recognizable inherited variables basic to an understanding of the genetics of the species and necessary to any research relating to the discipline of heredity. Consequently, unrelated strains of C. tarsalis from different geographic areas have been collected and colonized in a search for mutants. In addition to screening various strains from neighboring Kern County and Sacramento Valley areas in California, we now have laboratory colonies from Yuma, Arizona, Ft. Collins, Col., Imperial Valley, Cal., and Winnipeg, Canada. At this writing we are also awaiting receipt of specimens from 2 different areas in Texas and another from Canada. Another strain that we maintain in our laboratory is in complete opposition to these highly inbred lines. This is a composite strain that holds some of the genotype from each of our colonies. "Left-overs" from other strains contributed to its origin. This strain has good hybrid vigor and is therefore useful as a research organisms. Because of its broad gene pool it should also serve as an excellent line for physiological studies since inbred lines are thought by many to be poor representatives of a species, especially for physiological experiments.

The mutations that have to date been successfully isolated and established for the purpose of constructing marker stocks are summarized in Table 1.

## B. Cytogenetics

At the time of the original application a paper, "Cytogenetic observations in Culex tarsalis: Mitosis and Meiosis" was in press. It was published in J. Med. Ent., July 1974, and is a study of the karyotype and behavior of chromosomes in mitotic and meiotic cycles in sequential divisions to ascertain normal patterns in spermatogenesis. Such information is basic in light of the current interest in using induced chromosomal aberrations as possible tools for population control, primarily since, unfortunately, salivary gland chromosomes of culicine mosquitoes cannot be successfully used for detailed recognition of abnormalities at the present time. (Reprints inclosed)

### Reciprocal translocations

Following radiation treatment with approximately 4000 r. young male adults commonly father progeny in which a goodly number of the embryos are inviable. Cytological examinations of the primary spermatocytes of viable offspring have shown that in some cases this reduction in egg hatch is due to reciprocal translocations involving 2 of the 3 chromosomes. At the present time, 7 different lines carrying such a heritable aberration responsible for embryo lethality have been established in the laboratory. While lack of a genetic marker strain inhibits the making of necessary crosses that would identify the location of the breaks and recombination points, cytological observations identify 5 of these interchanges involve the 2 autosomes and 2 involve the sex-determining chromosome and one of the larger pairs. To our knowledge this is the first study of this type with C. tarsalis. A paper describing the cytological evidence of these interchanges is in preparation. Since the mechanics and dose that led to these interchanges are now well established, no further ones will be induced until marker strains are constructed with our mutants for identification and screening purposes.

### Inherited polyploidy

Another interesting line recently isolated from a stock carrying a reciprocal 2-3 translocation seems to carry a mutant gene (or genes) responsible for polyploidy and eventual lethal spermatids. This characteristic has remained in this stock for over 20 generations and undoubtedly contributes to the low egg hatch in the colony. While no one pattern can be identified, indications are that the mutation interferes with normal Anaphase I in spermatogenesis, giving rise to a large number of imbalanced sperm. Since the characteristic persists from generation to generation, it is assumed at this point that the gene (genes) behave as a recessive mutant carried in the gene pool of the stock. In the homozygous condition lack of normal meiotic behavior initiates abnormal segregation causing cells to be tetraploid, triploid, or aneuploid in a variety of ways for 1 or more of the 3 pairs of chromosomes. More study will be needed, but we may have another autocidal system that could be carried into a native population with a heterozygous or homozygous translocation as the vehicle.



### C. Refractoriness of *Culex tarsalis* to WEE virus infection and/or transmission.

One of the potential uses we envision for our translocations is to transport desirable genotypes into a native population--e.g., a male-producing mechanism, temperature-lethal gene, or possibly a genotype that would interfere with the status of *C. tarsalis* as a vector of WEE or SL virus. We are investigating one such genotype in collaboration with Dr. James Hardy, School of Public Health. For several years it has been noted that individual females from different geographic field populations, as well as females within some strains that we already have, vary considerably in their susceptibility to infection with WEE virus. More recently we have observed that this variability also exists for the ability to transmit the WEE virus once a female is infected. Assuming that both of these factors have a genetic basis we have been selecting lines that are susceptible or refractory to virus infection, and lines that are able or unable to transmit virus once infected. While we have encountered problems, especially after inbreeding for several generations, our selection process has continually increased for the desired genotypes. After 7 generations we have data to show that up to 70% of the progeny of individual egg batches are refractory to WEE virus infection after feeding on a high concentration of virus. We have another geographic strain in which the females are routinely 100% susceptible. Another line is I+T-, that is, 100% infectable but routinely some of the infected females cannot transmit the virus to chicks after the normal or even prolonged incubation periods. In single-pair matings of this strain we have recovered offspring where 60% of the female progeny cannot transmit the virus after being successfully infected on high concentrations of virus by plegget feeding. This inability to transmit appears to be related to a low level of virus found in the bodies of such females. At the present the inability of virus increase in these females is considered to be due to a "gut barrier". If the gut is bypassed and the virus is directly injected into the hemocoel, all individual females become and stay infected at the end of the incubation period. What causes this "barrier" to inhibit virus replication and thus ultimately render the female unable to transmit is not yet understood. Dr. Edward Houk an insect physiologist who has joined the project (see added personnel) is presently initiating a study to determine the level where the barrier is operating. He hopes to gain some insight by studying the differences between *Culex peus* and *C. tarsalis* in the gross structure of their guts and in related bio-chemical differences, as *Culex peus* cannot be infected by feeding but can be infected by injection.

### D. Autogeny in *Culex tarsalis*.

Autogenous mosquitoes have less contact with hosts and are consequently less efficient vectors. Such extrinsic factors as photoperiod, temperature, and food have a major influence over the expression of autogeny; however, a genetic basis has also been recognized. Autogeny in *C. tarsalis* was first reported in 1958, and since then several strains have been successfully established with varying degrees of autogeny. Strictly anautogenous strains also occur. Preliminary studies by Moore (1966) suggested that autogeny was dominant to anautogeny; however, no thorough genetic study has been conducted. In closely related *C. pipiens* (Spielman, 1974) autogeny seems

to be under the control of at least 2 genes, although the situation is not clear cut, perhaps because the stocks used were polymorphic. Dr. Paul McDonald, our post-doctoral associate, has established inbred autogenous and anautogenous lines, and is now in the process of making the first genetic crosses. If the inheritance of autogeny is simple, we will study seasonal polymorphisms. Ultimately we would consider the incorporation of autogeny into vector incompetent strains for release in control programs, as there is reason to believe that autogeny delays the taking of a first blood meal. Any delay in blood taking should have a marked effect in suppression of virus transmission.

#### E. Diapause in *Culex tarsalis*.

The ability or failure to enter diapause is an important characteristic useful in a genetic control program. We are searching for inability to diapause by stressing *C. tarsalis* strains obtained from consistently warm areas. In nature these populations probably do not go into diapause. If we are able to isolate these populations and determine their genetic basis, then we would be able to consider their usefulness for control in areas of the mosquito's range which requires a seasonal diapause for survival. The ability to stimulate diapause in the laboratory would also be useful as a means of putting females into a holding stage until eggs and progeny are needed, and replace present techniques of continual maintenance.

Dr. McDonald has considerable data to indicate that the critical time to trigger diapause is in the 3rd or earlier larval stage since shortening the day-length to 8 hours resulted in adult females developing physical characteristics comparable to diapausing females in nature--extensive fat bodies and general inactiveness. Dr. McDonald is also presently comparing field collected diapausing females and those assumed to be in that state under laboratory-reared conditions. Another experiment in process deals with differences between autogenous and anautogenous females in response to changes in the photoperiod during immature instars.

#### F. Mating behavior.

Two concerns relate to density dependent factors in release programs for control purposes. These are whether or not the females in question can be inseminated more than once, and the number of times a single male would be apt to successfully inseminate females. Using normal and genetically marked individuals (our black-eyed mutant), 40 pint-size cages were prepared, each holding a single black-eyed mutant female and two males--one with normal eye color and one carrying the black-eyed marker. Both female and males were 3 days old and had been reared under the same conditions. After a period of 7 days the 40 anautogenous females were given a blood meal. Thirty-three egg rafts were recovered and reared separately. All emerging adults were observed for eye color. Since the black-eyed mutant behaves as an autosomal recessive, the adult  $F_1$  progeny would show: a) normal eye color if the female had been inseminated by a normal-eyed male only; b) black eye, if the female had been inseminated by a black-eyed male only; c) a mixture of normal and black-eyed individuals if the female had been inseminated by both males. The results can be seen in Table 2.



While the data does not rule out the possibility of dual insemination in nature or over a long-time period, it does indicate that generally the female will be successfully inseminated only once within a time period of one week. This is in accord with other culicine mosquitoes that have been tested for this factor.

To determine how frequently a single C. tarsalis male can inseminate virgin females of specific age and within specified time periods, 2 separate experiments were set up in the following manner. Using young (12-24 hrs) adults, 42 cages were set up in a ratio of 10 females to 1 male. To ascertain if size of cages affected the mating behavior, 21 cages were of pint size and 21 were of gallon size. For 7 consecutive days thereafter, the females of 6 cages--3 pint and 3 gallon--were removed and anesthetized. Spermathecae were dissected to observe the presence of sperm. Two tests were run using adults from 2 different laboratory strains--UC and the Owen's Valley stock. The highest number of females inseminated by a single male in the three cages for each strain was recorded. The results are given in Table 3.

In the second experiment, 21 gallon cages were prepared holding 10 females and 1 male of older age (72-84 hrs.), since it was obvious from the above test that adults could not successfully mate if one sex or both were younger than 3 days. Each day the females from 3 cages were removed and observed for sperm in the spermathecae. To see if there was any recovery of reproductive potential once a depletion of sperm might have occurred, males that were caged with females for 5, 6, and 7 days were given 10 new 3-day-old virgin females once the first batch was removed. These females were again left for 5, 6, and 7 days. All females were dissected to observe for the presence of sperm. The results are shown in Table 4.

From the data obtained it can be seen that the highest number of females that was inseminated by a single male in a 14-day period was 8. This is not necessarily the case in nature, but gives some indication of male mating behavior under specific conditions.

#### G. Temperature and embryonation delay in *Culex tarsalis*.

One disadvantage in using Culex species for research purposes is the inability to control egg hatch, especially in autogenous strains where blood meals are not required. While it is known that in nature females of C. tarsalis go into diapause in the late fall where climatic conditions initiate such an over-wintering phase, the environmental factors that will duplicate this mechanism in the laboratory are not understood. Thus for the present, development of alternate holding stages to prevent uncontrolled development in this particular species would be useful. It seemed reasonable to attempt to store eggs at a reduced temperature to delay embryonation and egg hatch.

Single egg rafts of similar age (5-10 hrs.) and of the same laboratory strain were allowed to embryonate 5 to 10, 29 to 34, and 53 to 58 hrs. in open vials of water at room temperature before being stored at 45° F. In each test, 3 egg batches were kept at normal rearing temperature (72° F.) as controls. Every 24 hrs, for 6 consecutive days, 3 isolated egg rafts were removed from the colder environment to 72° F. The percent hatch of

each raft was recorded. After hatching, a specified number first-instar larvae--usually 150 unless low hatch made this impossible--were reared in the normal manner. Emerging adults were counted to ascertain any adverse effects in continued development due to cold and/or the interruption in embryonation. The UC strain was used and the test was repeated 3 times. Since the results were similar in the 3 trials, only 1 set of data is given in Table 4. The percent emerged did not favor 1 sex, so the adult number is not separated for sex.

From the data (Table 5) it appears that eggs of C. tarsalis may be stored at 45° F. for 1 to 3 days and still give 30 to 80% of adults depending on several factors. The shorter the embryonation time prior to cold storage the greater seems to be the chance for first-instar larvae to develop normally once a favorable temperature is restored. Similarly the length of time in a cold environment not only affects the percent of egg hatch but also interferes with continued development to the adult stage. There appeared to be no appreciable delay in time of egg hatch or time of development to adults once the eggs were returned to normal rearing temperature. Among the progeny surviving the hatching process, the greatest amount of mortality was seen in the late pupa stage and emerging process. Storage for 3 days would have some utility in delaying the necessity for maintenance of successive generations, but is not sufficient to resolve the basic need to be able to hold a generation in some stage for periods of one or more months.

#### H. Mass rearing and release factors.

Initial field experiments were done during August and September of 1974 by collaborators to study the feasibility of substituting the seeding of eggs into semi-natural breeding areas of the wild populations for the mass production of adults in laboratories. The mechanics of building up laboratory colonies to harvest large numbers of egg rafts at one time were successful, and as many as 1,000,000 eggs were produced in a week period using a 2 cubic-foot cage. Outdoor rearing trays were constructed and were seeded with egg rafts. Various diets and methods of feeding the hatched larvae were tried, and emerging adults were collected and counted. In the most successful tests over 10,000 adults were recovered from a 24 square-foot unit. The data will help give direction to new studies in the next spring and summer. Such a method of seeding populations will cut down considerably on the physical conditions necessary for mass rearing of adults for release programs.

### III. Proposed Studies for the Coming Year

A knowledge of the genetic variability of a mosquito species is basic for an understanding of the genetics of the organism as well as to other areas of its applied biology. For this reason the search for visible mutants affecting the more obvious structures or behavior of C. tarsalis will continue. Such findings will contribute to the description of new genes, and to the formal genetic information of this species. Some of the mutants already described will be useful as "tracers" or marker genes as already demonstrated in the experiment on mating behavior described above. Those



Table 1. MUTANT STOCKS of Culex tarsalis (June, 1975)

1. ble: black-eye: Spontaneous, isolated from a field-collected strain in Kern Co., Cal. Produces a shiny black superficial pigment, resembling a black oily film. Good penetrance in both sexes. Recessive and autosomal (II or III).
2. car: carmine-eye: Spontaneous, isolated from progeny collected in Yuma, Arizona. Eyes velvety red in larvae, pupae and young adults, bright color fading with age in the latter. Recessive and autosomal but not linked to ble (II or III). Good penetrance in both sexes.
3. mul: mulberry-eye: Induced with EMS (ethyl methane sulfonate). Facets of compound eye irregular in shape. Good penetrance in both sexes. Recessive and sex-linked (I).
4. ble/car: black-carmine eye: Marker stock for two autosomes. Good penetrance in both sexes, with both pigments expressed simultaneously. Individuals homozygous for both mutants have carmine eyes as larvae and pupae; as young adults the anterior portion of the eye is carmine and the posterior portion is black. As the adults age, the eye is typically as the ble mutant, and car cannot be recognized. Recessive to wild-type.
5. mul/ble/car: mulberry, black-carmine eye: Complete marker stock with one mutant on each of the 3 linkage groups. Good penetrance of all 3 phenotypes in the compound eye of the same individual. In addition to the 2 pigment characteristics described above, the eye expresses the mulberry mutant (mul).
6. fri: fringed-wing: Radiation-induced. Scales of wing ruffled both on surface and edge, giving impression of a fringe on wing. More pronounced in females, but easily recognized in both sexes. Recessive and sex-linked.

Table 2. Eye color of progeny from a black-eyed female caged with a normal and a black-eyed male.

	Inviabile eggs	All progeny with + type eye	All progeny with black eyes	Progeny mixed in eye color
Number of rafts	4	16	13	0

Table 3. Highest number of females inseminated by a single male over specific time periods. \*

Strain	Cage size	Days of association						
		1	2	3	4	5	6	7
Owen's Valley	Pint	0	0	2	2	5	4	5
UC, Berk.	"	0	0	1	2	4	4	4
Owen's Valley	Gallon	0	0	1	2	3	3	4
UC, Berk.	"	0	0	4	3	5	5	7

\* Each cage contained 10 females and 1 male--both sexes 12-24 hrs. old.

Table 4. Highest number of females inseminated by a single male over specific time periods. \*

	cage size	Days of association						
		1	2	3	4	5 + 5	6 + 6	7 + 7
No. of females inseminated	Gallon	2	4	3	5	5 2	4 2	6 2
						(7)	(6)	(8)

\* Each cage contained 10 females and 1 male of the UC strain....both sexes 72-84 hrs. old. Days 5, 6, and 7 totaled 20 females.

Table 5. Egg hatch and development in Culex tarsalis after various intervals of egg storage at 45° F.

Age of eggs prior to storage	Storage time (days)	% egg hatch *	% of hatched larvae reaching adulthood
5-10 hrs	0	98	81
	1	100	64
	2	93	70
	3	95	65
	4	90 **	25
	5	82 **	2
	6	0	0
29-34 hrs	0	99	79
	1	90	70
	2	93	41
	3	36 **	26
	4	51 **	31
	5	0	1
	6	0	0
53-58 hrs	0	100	72
	1	96	43
	2	60	32
	3	63	35
	4	15 **	9
	5	17 **	23
	6	0	0

\* Average of 3 egg rafts

\*\* Many died after hatching



# VI. Updated biographical sketch and bibliography (principal investigator)

## A. Presented papers

"Genetics of the Encephalitis Vector, Culex tarsalis, for possible application in integrated control" 1974 Meeting of the Northern Calif. Parasitologists, Asilomar, Pacific Grove, Calif.

"Genetics of New Mutants in Culex tarsalis" 1975 Meeting, Calif. Mosquito Control Assoc. Redding, Calif.

## B. Symposiums (invitational papers)

"Current Status of Genetic Control of Mosquitoes" (Panel with R. A. Barr, A. McClelland and P. McDonald) 1974 Calif. Mosq. Cont. Assoc.--Entomology Seminar, Fresno, Calif.

"Current Status of the Genetics of Culex tarsalis" Symposium: Genetics of Mosquito Populations, 1974 ESA Meeting, Minneapolis, Minn.

## Publications

Asman, S. M. 1974. Cytogenetic observations in Culex tarsalis: Mitosis and Meiosis. J. Med. Ent. 3, 375-382.

Asman, Sr. M. 1974. Induced translocation in Culex tarsalis for possible use in control systems. Proc. Amer. Mosq. Cont. Assoc. Vol. 42, 168 (abstract)

## To be submitted in February, 1975

Asman, Sr. M. "Two examples of Mating Behavior in Culex tarsalis"--to Annals of the Entomol. Soc.

Asman, Sr. M. "Temperature and embryonation delay in Culex tarsalis"--to Mosquito News

## In preparation

Asman, Sr. M. "Four new eye mutants in Culex tarsalis"

Asman, Sr. M. "Radiation-induced reciprocal translocations in Culex tarsalis"



VII. New personnel (Biographical Sketch)

Name: Paul T. McDonald

Title: Postgraduate Research Entomologist VI

Birthdate: October 15, 1940

Birthplace: Waterbury, Connecticut

Citizenship: U. S.

Education: B.A. (Biology), Yale College, New Haven, Conn., 1964  
Ph.D. (Biology), University of Notre Dame, South Bend, Ind., 1970  
Studies in education, Fairfield University, Fairfield, Conn.  
1965-1966.

Postdoctoral Activities:

Research Associate under K.S. Rai, U. Notre Dame, 1970-71.

Research Fellow of International Centre of Insect Physiology  
and Ecology at Mosquito Biology Unit at Mombasa, Kenya, 1971-74.

Publications:

P. T. McDonald and K.S. Rai. 1970 Aedes aegypti: Origin of a "New"  
chromosome from a double translocation heterozygote. *Science*  
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